© Comparative Cytogenetics, 2009 . Vol. 3, No. 1, P. 63-69. ISSN 1993-0771 (Print), ISSN 1993-078X (Online)

Main trends of karyotype evolution in the superfamily Chalcidoidea (Hymenoptera)

V.E. Gokhman[1], A.V. Gumovsky[2]

[1]Botanical Garden, Moscow State University, Moscow 119991, Russia. [2]Institute of Zoology, National Academy of Sciences, Kiev 01601, Ukraine.
E-mail: [1]gokhman@bg.msu.ru, [2]gumovsky@izan.kiev.ua

Abstract. An overview of karyotype evolution in the superfamily Chalcidoidea is given, including new data on karyotypes of *Eurytoma curculionum* Mayr, 1878 (Eurytomidae; 2n=20) and *Euderomphale chelidonii* Erdös, 1966 (Eulophidae; n=6). Structural types of chromosome sets in the superfamily Chalcidoidea are listed. Main pathways of karyotypic change in the Chalcidoidea are outlined. The chromosome set containing eleven subtelo- or acrocentrics is considered as an ancestral karyotype for the superfamily. Multiple independent reductions in "n" values through chromosomal fusions presumably occurred in various groups of chalcid families.

Key words: chromosomes, karyotypes, parasitic wasps, Chalcidoidea.

Introduction

The superfamily Chalcidoidea is a large group of parasitic wasps comprising approximately 22 thousand described species (http://www.nhm.ac.uk/research-curation/research/projects/chalcidoids/introduction.html,on May 4, 2009); however, chromosomes of only about 160 species are known up to now (see Gokhman, 2009 for review). Nevertheless, a few preliminary reconstructions of karyotype evolution in the superfamily were suggested during the last years (e.g. Gokhman, 2004). Moreover, recent results of chromosomal analysis of certain groups of Chalcidoidea provide new information regarding the existing pattern of karyotypic variation within the entire superfamily as well as at the family level (Fusu, 2008a, 2008b; Gokhman, Mikhailenko 2008; Gokhman et al., in press). The results of this reanalysis are given below.

Material and Methods

The discussion presented in this paper is mostly based on the results of the recent review of karyotypes of parasitic wasps (Gokhman, 2009). However, information from a few papers that could not be included in this review for technical purposes (e.g. Fusu, 2008b) is also considered. Original data used in the present paper were obtained from chromosomal study of the two additional species of the superfamily Chalcidoidea, namely, *Eurytoma curculionum* Mayr, 1878 (Eurytomidae) and *Euderomphale chelidonii* Erdös, 1966 (Eulophidae; both identified by the second author). These species were selected to illustrate the concept of the so-called "low-numbered" and "high-numbered" families of the Chalcidoidea (see below). Hosts of parasitic wasps, *Rhinusa asellus* (Gravenhorst, 1807) (Coleoptera: Curculionidae) and *Aleyrodes proletella* (Linnae-

us, 1758) (Hemiptera: Aleyrodidae) respectively were collected by A.V. Gumovsky in December 2008 in Kiev (Ukraine). Chromosomal preparations were made from cerebral ganglia of prepupae extracted from the hosts (see Imai et al., 1988 for the detailed description of the karyotyping technique). Digital images of metaphase plates were obtained using Zeiss Axioskop 40 FL light microscope equipped with AxioCam MRc camera. To prepare karyograms, the resulting images were processed with Adobe Photoshop version 8.0. Chromosomes were arranged on karyograms according to Levan et al. (1964).

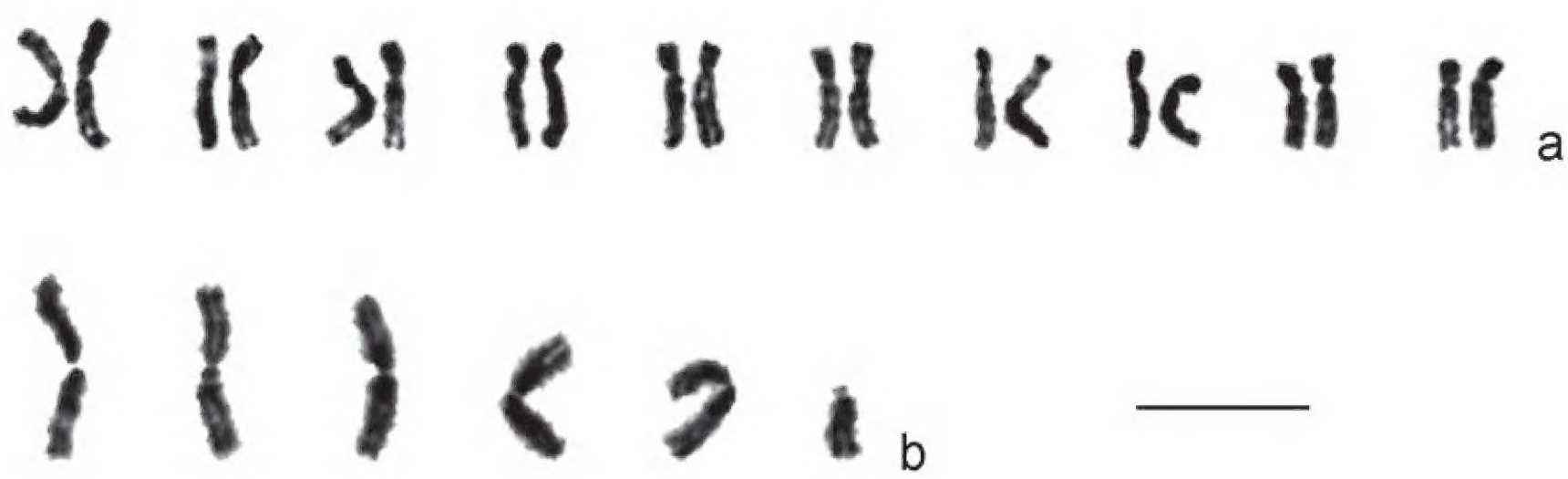

Fig. 1, a, b. Mitotic karyograms of Chalcidoidea: **a** - *Eurytoma curculionum*, female. **b** - *Euderomphale chelidonii*, male. Bar = 10 μm.

Results

Eurytoma curculionum (Fig. 1, a). 2n=20. All chromosomes of the female karyotype of this species are biarmed and more or less gradually decrease in size (probably except for the first and two last pairs). Metacentrics of the first pair are the largest elements of the chromosome set. Other chromosomes of the karyotype are subtelocentrics (presumably those of the fourth, seventh and eighth pairs) and submetacentrics (the remaining ones).

Euderomphale chelidonii (Fig. 1, b). n=6. The male karyotype of this species contains five large metacentrics (first three chromosomes are slightly longer than the others) and a smaller acrocentric.

Discussion

To understand the process of chromosomal evolution in the Chalcidoidea, which is considered monophyletic (following Gibson, 1986, 1999; Gibson et al., 1999; Campbell et al., 2000; Desjardins et al. 2007), it is necessary first to reconstruct chromosome numbers and other karyotypic features that could be considered putatively ancestral for the whole

To assess the pattern of karyotype evolution in the superfamily, the principal phylogenetic scheme developed by Noyes (1990) is used. However, Pteromalidae are represented on the scheme only by their least specialized subfamily, Pteromalinae; phylogenetic relationships of the families Rotoitidae, Tetracampidae, Trichogrammatidae and Eulophidae are shown according to Gumovsky (2008). Since Pteromalidae, Tetracampidae and Aphelinidae are obviously not monophyletic (Campbell et al., 2000; Gumovsky, 2008), their relationships with other groups are shown by dashed lines.

superfamily. One of the possible approaches to detecting those n values is to superimpose the most common chromosome numbers onto the phylogenetic tree of superfamilies of parasitic wasps. Since Chalcidoidea represent a terminal branch in the so-called Microhymenoptera lineage comprising Diaprioidea, Cynipoidea, Platygastroidea and Ceraphronoidea (Ronquist et al., 1999), and most frequent haploid chromosome numbers in this clade vary from 9 to 11 (see Gokhman, 2009), we therefore presume that these n values can be ancestral for the Chalcidoidea as well.

Indeed, the so-called "high-numbered" families do present within the Chalcidoidea, i.e. Mymaridae (n=9) and Encyrtidae (n=8-12). In addition, many species of Eurytomidae and Aphelinidae also have n=9-11 (see e.g. Fig. 1, a), and n=10 have been recently detected in the family Torymidae where the majority of species have n=4-6 (Fusu, 2008a). Moreover, parasitic wasps with n=7-10 have been found among Eupelmidae, which generally have n=5 (Fusu, 2008b). On the other hand, most chalcid families (Agaonidae, Chalcididae, Eulophidae (with an exception of *Elachertus* sp. with n=8), Leucospidae, Ormyridae, Perilampidae, Pteromalidae and Trichogrammatidae) have n=3-7 and are therefore "low-numbered" (see e.g. Fig. 1, b; Gokhman et al., in press). Nevertheless, at least Mymaridae are usually treated as a sister group to the rest of Chalcidoidea (Gibson, 1986, 1999; Gibson et al. 1999; Campbell et al. 2000; Desjardins et al. 2007), and higher chromosome numbers (n=9-11) can therefore be considered initial for the superfamily due to the outgroup presumption (Gokhman, 2009).

We suggested earlier (Gokhman, Quicke 1995) that n=3-7 found in the "low-numbered" families of Chalcidoidea could represent their synapomorphy. However, more detailed phylogenetic analysis of chromosomal variation within the superfamily does not support this assumption (Gokhman, 2004, 2009; see also Fusu, 2008a). On the contrary, multiple independent reductions in chromosome number from n = 9-11 to n=3-7 occurred at least in three groups of families, namely: (Trichogrammatidae + Eulophidae) (perhaps also in Rotoitidae and Tetracampidae), ((Pteromalidae + (Eucharitidae + Perilampidae)) + ((Ormyridae + Torymidae) + Agaonidae)) as well as in the (Chalcididae + Leucospidae) (Fig. 2; chromosomes of the family Eucharitidae are currently unknown). In addition, chromosome number also decreased at least twice in the Aphelinidae where many species have n=8-11; however, the subfamily Aphelininae and some species of the genus *Encarsia* (Coccophaginae) have n=3-7 (see data in Gokhman, 2009). An analogous reduction in this parameter has occurred in two related species groups of the genus *Eurytoma* (n=5-7) while all other Eurytomidae have n=9-10 (Gokhman, Mikhailenko, 2008). A more complicated situation can be observed in the family Eupelmidae where the majority of species have n=5; nevertheless, three species belonging to different genera have n=7, 8 and 10 (Fusu, 2008b). Close relationships between Eupelmidae and Encyrtidae remain questionable (Campbell et al., 2000); however, these groups are supposedly related due to a few common morphological features (e.g. shape of the acropleuron; Noyes, 1990). We therefore suggest that the most frequent chromosome number in the Eupelmidae, n=5, independently arisen at least twice within two structurally different karyotypes (in *Eupelmus* and related genera as well as in *Anastatus*) from those with n values about 10, and the reverse opinion recently expressed by Fusu (2008b) is incorrect if Encyrtidae are used as an outgroup. We believe that higher chromosome numbers can be supposed as derived only in the family Torymidae generally

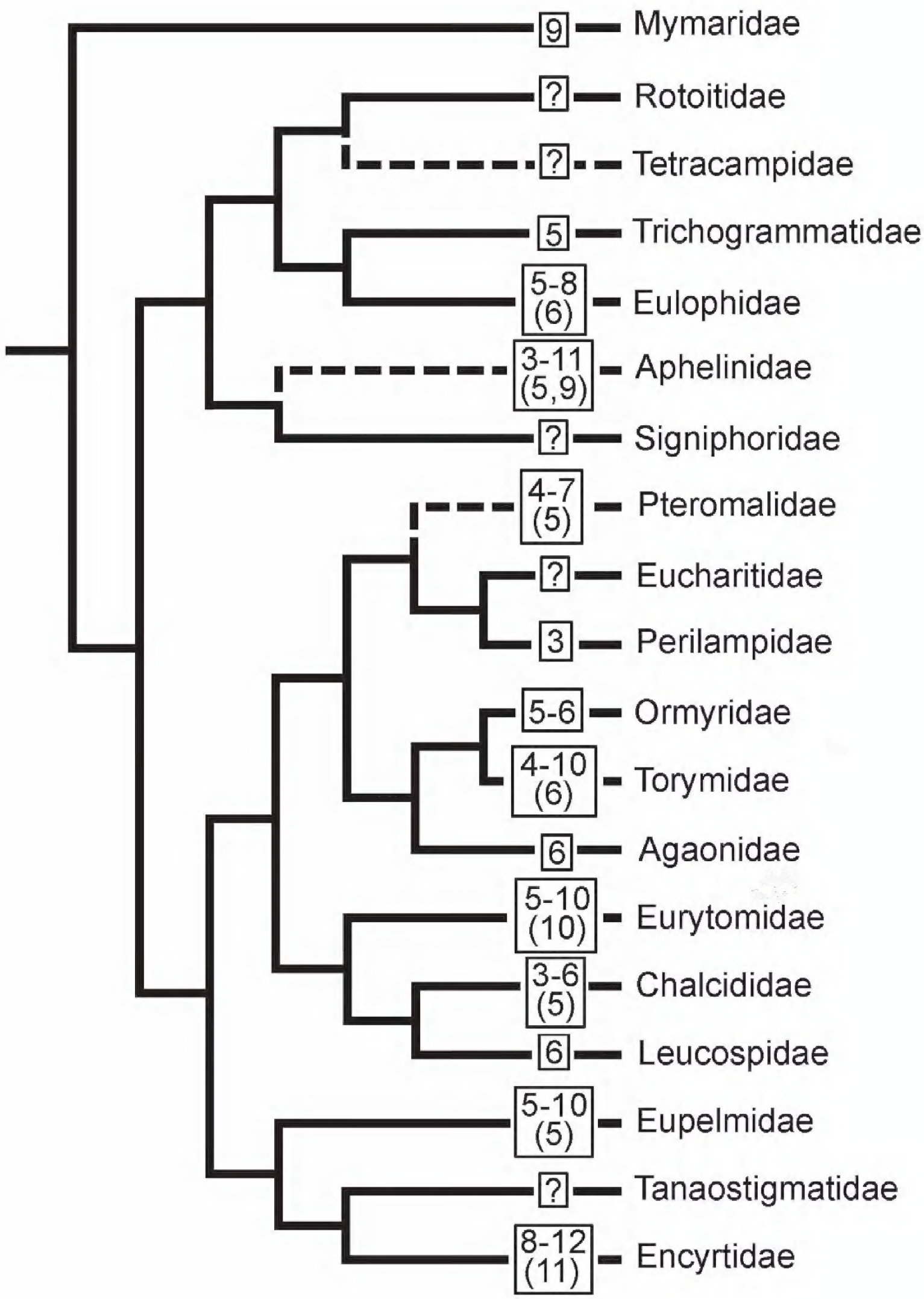

Fig. 2. Phylogenetic tree of chalcid families (based on Noyes, 1990; see the text for further explanations) with ranges of haploid chromosome numbers superimposed onto it (based on data from Gokhman, 2009 and recent updates by the first author). Most frequent "n" values are given in brackets.

having n=4-6, where they are characteristic only of the advanced genus *Podagrion* (n=10; see Fusu, 2008a).

The described pattern does not change substantially if not only chromosome number, but chromosomal morphology as well is taken into account. Thus, higher chromosome numbers (n=10-11) are usually associated with abundance of subtelocentrics and acrocentrics within the karyotype (e.g. in the Encyrtidae, Aphelinidae and Eurytomidae). On the other hand, metacentrics and submetacentrics often prevail in chromosome sets of the Chalcidoidea with lower chromosome numbers. Moreover, two more or less distinct morphological types of karyotype structure can also be selected in the latter case, specifically: (1) all chromosomes are meta-/submetacentric, as e.g. in many Pteromalinae (Pteromalidae) with n = 5, and (2) most chromosomes are meta-/submetacentric within the haploid karyotype, but one (rarely two) chromosomes are subtelo-/acrocentric; these chromosomes are substantially smaller than meta-/submetacentrics (see Gokhman, 2009). However, chromosome sets of the second type are easily convertible into those of the first type through tandem fusions. In the superfamily Chalcidoidea, karyotypes with five large metacentrics and a smaller subtelo-/acrocentric can be found in many Torymidae, one of the two studied species of the Ormyridae and the only examined species of the Agaonidae, and are therefore considered the ground plan feature of these families (Gokhman et al., in press). Surprisingly enough, the same karyotype structure is also considered initial for the family Eulophidae (Gokhman, 2009) that belongs to the other clade of the Chalcidoidea. Nevertheless, this fact can be considered as a case of parallelism, since similar chromosome sets are also common in the unrelated subfamily Cheloninae (Braconidae; Gokhman, 2009; also see below). At lower taxonomic levels, chromosomal fusions also seem to prevail, especially in the Aphelinidae, Eurytomidae, Eulophidae, Encyrtidae and probably also Eupelmidae.

Due to relatively poor knowledge of phylogenetic relationships between chalcid families, it is difficult at present to reliably determine an ancestral karyotype structure for the Chalcidoidea. However, accumulated data suggest that the ancestral haploid karyotype of the superfamily could contain eleven subtelocentric or acrocentric chromosomes that gradually decreased in size. Current analysis shows that chromosome sets of that kind could be inherent for the ancestors of at least two, putatively unrelated chalcid families, namely, Aphelinidae and Encyrtidae. Moreover, karyotypes of some Eurytomidae (e.g. certain species of the genus *Eurytoma*) could originate from the initial one through a single centric-like fusion. In addition, basal haploid karyotypes of the (Agaonidae + (Ormyridae + Torymidae)) as well as of the Eulophidae also contain eleven longer arms, and could therefore result from the two independent consequent processes of pairwise centric fusion within the initial chromosome sets. Finally, karyotypes of some Diaprioidea and Cynipoidea also have n=11 and contain considerable amounts of subtelo-/acrocentrics (Gokhman, 2009). However, two studied species of Mymaridae, the most basal family of the Chalcidoidea (following Campbell et al., 2000), have n=9. Nevertheless, both these species belong to the derived genus *Anaphes* (Huber, 2002), and further studies will probably provide new information concerning karyotypic variation in the Mymaridae.

In conclusion, it is also worth mentioning that among the two trends of karyotype evolution characteristic of the parasitic wasps as a whole, specifically: (1) decrease in chromosome number, and (2) karyotypic dissymmetrization (i.e. an increase in the size differenti-

ation of chromosome and/or in the proportion of acrocentrics in the karyotype; Gokhman, 2009), only the first one seems to be widespread in the Chalcidoidea. On the contrary, karyotypic dissymmetrization is substantially limited in its occurrence, and can usually be observed at initial stages of consequent fusion processes (e.g. in the genus *Eurytoma*; Gokhman, Mikhailenko, 2008).

Conclusion

Four main morphological types of chromosome sets are defined in the superfamily Chalcidoidea, specifically: (1) eleven subtelo-/acrocentrics that more or less gradually decrease in size; (2) a large metacentric and nine subtelo-/acrocentrics; (3) five large metacentrics and a smaller subtelo-/acrocentric; and (4) five large metacentrics. Chromosome set of the first type is presumed to be an ancestral karyotype for the Chalcidoidea. Other types of chromosome sets independently originated within various groups of the superfamily.

Acknowledgments

The authors are grateful to A.P. Mikhailenko (Botanical Garden, Moscow State University) for technical assistance. The present study was partly supported by the research grant no. 07-04-00326 from the Russian Foundation for Basic Research to VEG and a research grant from Alexander von Humboldt Foundation to AVG.

References

Campbell B., Heraty J., Rasplus J.-Y., Chan K., Steffen-Campbell J., Babcock C. 2000. Molecular systematics of the Chalcidoidea using 28S-D2 rDNA, (pp. 59-73) // Austin A.D., Dowton M. (Eds). *Hymenoptera: evolution, biodiversity and biological control.* Collingwood. 468 p.

Desjardins C.A., Regier J.C., Mitter C. 2007. Phylogeny of pteromalid parasitic wasps (Hymenoptera: Pteromalidae): Initial evidence from four protein-coding nuclear genes // *Mol. Phylogenet. Evol.* 45(2): 454-469. (doi:10.1016/j.ympev.2007.08.004.)

Fusu L. 2008a. Chromosomes of two *Podagrion* species (Hymenoptera, Chalcidoidea, Torymidae) and the evolution of high chromosome numbers in Chalcidoidea // *An. Ştiinţ. Univ. "Alexandru Ioan Cuza" Secţ. Genet. Biol. Mol.* 9(3): 61-64. (http://www.bio.uaic.ro/publicatii/anale_biochimie/2008_IX_F3/09_LFusu.pdf).

Fusu L. 2008b. The usefulness of chromosomes of parasitic wasps of the subfamily Eupelminae (Hymenoptera: Chalcidoidea: Eupelmidae) for subfamily systematics // *European J. Entomol.* 105(5): 823-828. (http://www.eje.cz/pdfarticles/1403/eje_105_5_823_Fusu.pdf).

Gibson G.A.P. 1986. Evidence for monophyly and relationships of Chalcidoidea, Mymaridae, and Mymarommatidae (Hymenoptera: Terebrantes) // *Canadian Entomol.* 118(3): 205-240.

Gibson G.A.P. 1999. Sister-group relationships of the Platygastroidea and Chalcidoidea (Hymenoptera): an alternate hypothesis to Rasnitsyn (1988) // *Zool. Scr.* 28(1-2): 125-138. (doi:10.1046/j.1463-6409.1999.00015.x).

Gibson G.A.P., Heraty J.M., Woolley J.B. 1999. Phylogenetics and classification of Chalcidoidea and Mymarommatoidea – a review of current concepts (Hymenoptera, Apocrita) // *Zool. Scr.* 28(1-2): 87-124. (doi:10.1046/j.1463-6409.1999.00016.x).

Gokhman V.E. 2004. Karyotype evolution in parasitic Hymenoptera // *Zool. Zh.* 83(8): 961-970. (In Russian, with English translation in *Entomol. Rev.* 84 (suppl.): S41-S49).

Gokhman V.E. 2009. Karyotypes of parasitic Hymenoptera. Dordrecht. 183 p. (doi:10.1007/978-1-4020-9807-9).

Gokhman V.E., Mikhailenko A.P. 2008. Karyotypic diversity in the subfamily Eurytominae (Hymenoptera: Eurytomidae) // *Folia Biol.* (Krakow). 56(3-4): 209-212. (doi:10.3409/fb.56_3-4.209-212).

Gokhman V.E., Mikhailenko A.P., Fursov V.N. (in press). Chromosomes of *Blastophaga psenes* (Hymenoptera: Agaonidae) // *J. Hymenopt. Res.*

Gokhman V.E., Quicke D.L.J. 1995. The last twenty years of parasitic Hymenoptera karyology: an update and phylogenetic implications // *J. Hymenopt. Res.* 4: 41-63.

Gumovsky A.V. 2008. Chalcidoid wasps of the families Eulophidae and Tetracampidae (Hymenoptera:

Chalcidoidea): morpho-biological features and phylogeny. D.Sci. Dissertation, Institute of Zoology, National Academy of Sciences of Ukraine. Kiev. 234 p. (In Ukrainian).

Huber J.T. 2002. The basal lineages of Mymaridae (Hymenoptera) and description of a new genus, *Borneomymar*, (pp. 44-53) // Melika G., Thuróczy C. (Eds). *Parasitic wasps: evolution, systematics, biodiversity and biological control.* Budapest. 480 p.

Imai H.T., Taylor R.W., Crosland M.W.J., Crozier R.H. 1988. Modes of spontaneous chromosomal mutation and karyotype evolution in ants with reference to the minimum interaction hypothesis // *Japanese J. Genet.* 63: 159-185.

Levan A., Fredga K., Sandberg A.A. 1964. Nomenclature for centromeric position on chromosomes // *Hereditas.* 52: 201-220.

Noyes J.S. 1990. A word on chalcidoid classification. *Chalcid Forum.* 13: 6-7.

Ronquist F., Rasnitsyn A.P., Roy A., Eriksson K., Lindgren M. 1999. Phylogeny of the Hymenoptera: A cladistic reanalysis of Rasnitsyn`s (1988) data // *Zool. Scr.* 28(1-2): 13-50. (doi:10.1046/j.1463-6409.1999.00023.x).

Received May, 15, 2009.
Accepted by V.G. Kuznetsova, June 16, 2009.
Published August 6, 2009